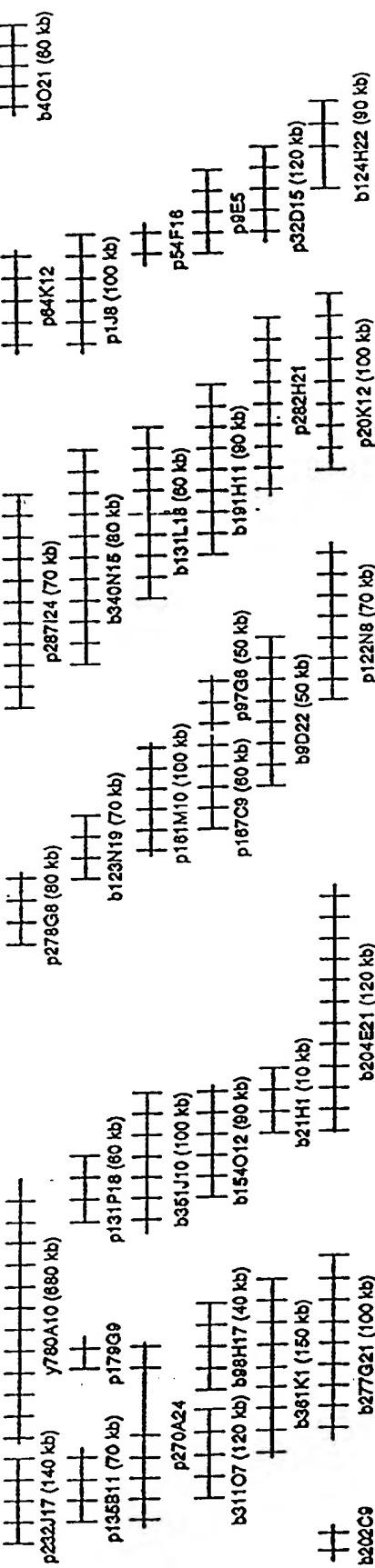


FIG. 1

UC-61 (GPC1), UCNP-20
yds5004
yds5007
UC-62
UC-63
UC-64, UCNP-19
ATSV-3, UCNP-14
UC-65
UC-66
UC-67
UC-68
ATSV-5
D2S140
AGXT, UCNP-21
UC-69
Z92102
UC-70
UC-71
UC-72 (W5812)
UC-73
UC-74, UCNP-17
UC-75
UC-76, UCNP-18
UC-77 (L866106)
UC-78
UC-79 (zmu1604), UCNP-13
UC-80
ST 16393-5, HS79337
HS79337, UCNP-12
ST 16393-3, HS79337, UCNP-10, 11
UC-82
UC-83
UC-84
HOLBP, UCNP-5, 16
UC-85
UC-86 (EST23509)
HS-112179, UCNP-8, 9
UC-87
UC-88
UC-89
WI-117474
UC-90
UC-91 (GM59407)
SGC2276, UCNP-6, 7
WI-15981
UC-92

FIG. 2



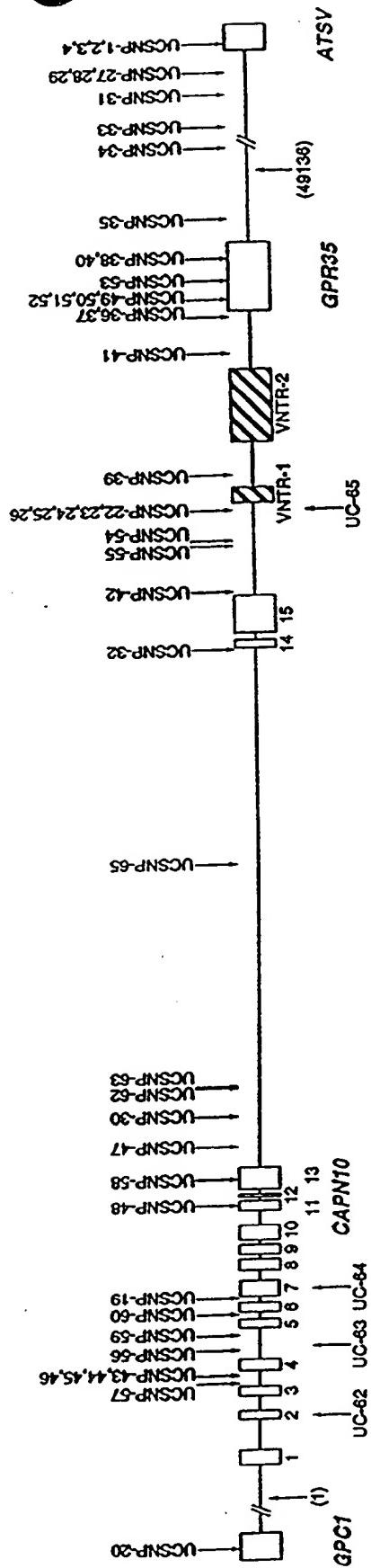


FIG. 3

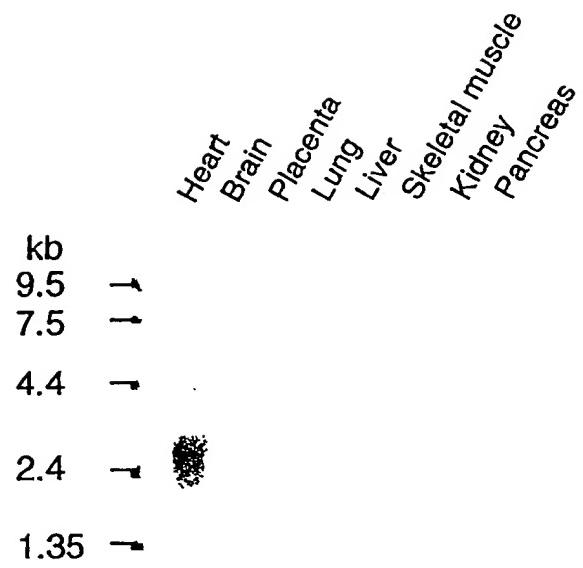


FIG. 4

		Domain I		Domain II	
BCAPN5		-MFSCVKPYEDQNYSLRQDCRRKVLFEDPLFPATDOSLYY-KGTPGP-		-AVRKWRPKGICEDPRLFVDC	67
BCAPN6		-MPPPLKLFIQKQYELKRCMHDGRFLCDPTFLPENDEFLFNRLPG-		-KVVWKRPKDIDSPDHLIVNG	67
BCAPN3	MPTVISASVAPRTAAEPRSGPVPHPAQSKEACCGNPSCIGSIAISRNFPPIYGKVKETKFLPEQFLKKCKLKVLYDVEPDFTPSLFSVQKPF-	-KARITHSSQSFEDQHREOCLQRGTLFEDADFPASNSLFLYSERQ-	-IQFWVKRPEPEICENPRFLIDG	115	
BCAPN9	KPYLY--	-RAPCPQAHPPV--		-IPFWVKRPGIEVKNPFEILGC	83
BCAPN1		-MSEIITPVYCTGSQAQVQKQARELGLC-	-RHENAALKYLGQDYEQLRVRCQLQSGTFLFDEAFFFPPVPSIYGKDLGPNSSKTYGIKWKRPTTELLSNQPFIVDG	101	
BCAPN2		-MAGLAALKADREAAEGLG-	-SHERAIKYLNQDYEAALRNCECLEATLFPQDFPSFPIASLGKELQGPPSSKTRGMWKRPTTEICADPQFIIQG	91	
BCAPN8		-MHALAAGVSKQRAVADGLC-	-SNQNAVQVYLQGDFTELTKQOCNLNSVGKLFDPDFPACPSALGYKGDLGPGSPDTGIVMKRPTTELCPNPQFIVGG	91	
BCAPN10			-KRAKGATPARELFRDAAFPAAQDSSLFCDSLSTPLAQFREDITWRRPQEICATPRLFPDD	59	
BCAPN5	ISSNDLHQGQVGNCHFVAACSSLASRESLMQKVTIPWKEQERIDPRKAQAYAGIFHFRFWRLC--MUVWVIDERLPTVNQMLIYCHSNRNEFWCALVEXAKAKLHGCYQALDGNTTADALV	186			
BCAPN6	ISNHOLIQGRILQKAKHISAFCSLQGQESWHTKAIPNNHQQEADPDKPKEYFHRFWLPGENTEVIDDLPLPTINGDOLVFSSTSNEFMSALLEKAYAKLHGCYEALDGLTTIDLI	187			
BCAPN3	ARTTDICQEGLCDCWLLAIALASLTNLQKALARVIP--	-HDQESEIENYAGIFHCFQWRYGENWDVUDIDCLPTYNQLVFTKSBNRNEFMSALLEKAYAKLHGSYEALKGNTTAE	229		
BCAPN9	ARTTDICQEGLCDCWLLAIALASLTNLQKALARVIP--	-QDQSFPGPYAGIFHCFQFWQHSENLQWVIDDRLPFTFDRRLVFLKSADENEFMSALLEKAYAKLHGSYEALKGSSAIE	197		
BCAPN1	ARTTDICQCALGDCWLAAIASLTNLQKALARVIP--	-HGQSOFQNYAGIFHCFQWQHSENLQWVIDDRLPFTFDRRLVFLKSADENEFMSALLEKAYAKLHGSYEALKGSSAIE	215		
BCAPN2	ARTTDICQCALGDCWLAAIASLTNLQKALARVIP--	-LAQSFQENYAGIFHCFQWQHSENLQWVIDDRLPFTFDRRLVFLKSADENEFMSALLEKAYAKLHGSYEALKGSSAIE	205		
BCAPN8	ARTTDICQCALGDCWLAAIASLTNLQKALARVIP--	-RDQSFQKDYAGIFHCFQWQHSENLQWVIDDRLPFTFDRRLVFLKSADENEFMSALLEKAYAKLHGSYEALKGSSAIE	205		
BCAPN10	PRECGQVKQGLGDCWFCLCACALQSRALLDQVTP--	-PCQPSWADQEQYRGSFTCR1WQFGRMVETTDDRLPCLAGRIFCSRCQEDVFMPLLEKTVAKVHGSYEHLMAQGVADALV	175		
BCAPN5	DFTGGVSEPIDLTERGDFANDETNRNQLFERMLKVKHSRGGLISASIKAV		TAADM	EARLACGLVKGKHAYAVITDV	258
BCAPN6	DFTCTLAEIYIDMQRGRYTDIWEKYKFLGELYKTFKGLLCCSIESP		SQEQ	EVEWTGGLLKGHTYMTDI	259
BCAPN3	DFTGGVAEEFEIRD--	-APSDMYKIMKJAIERGCSLNGCSIDDTGTRMTYGTSPSGLNGELIARMVRNMDNSLQDSULDFRGSDERPTTLPVYETRHAACGLVKGHAYSVTG	341		
BCAPN9	DFTGGVAETFQTRK--	-APENFYEILEKALKRGSLLLGCCFIDT	RSAES	EARTPPFGLIKGHAYSVTG	261
BCAPN1	DFTGGTWEYELRK--	-APSDLYQI1KALRKGSLLGCCSIDIS	S-VDW	EAITFKKLVLKGHAYSVTG	279
BCAPN2	DFTGGIAEWYELRK--	-PPPNFLKIIQKALQRGSLLGCCSIDIT	S-AADS	EAITFQKLVLKGHAYSVTG	269
BCAPN8	DFTGGISETFYDLRK--	-PPEMLYYIIQKALRKGSLLGCCSIDVC	T-AREA	EATTTRQKLVLKGHAYSVTG	269
BCAPN10	DUTGGLAEMWNLKGVAGSGCCQDREGRWHEHTRCQLLHLQDCLISCC		VLS	PRAGARELGEFHAFIVSDL	*
		Domain II		Domain III	
BCAPN5	RKVRLLGRGILLAFFKSEKLDMLMRNLPWGGEREWNGPMSDTSEEWQVSKSEREKGVTVQDDGEFWHMTFEDVCRYFTDIIKCRVINTSHL-SINKTWEEARL		HGAWTIHLEDPRQNRC	373	
BCAPN6	RKRLRGERLVEVFSTEKLVMVRRLNPLGRQEKSGPMSEISEEWNQQLTVTDRRKNLGLVMSDGEFVMSLDFCIRNFKHLNVCRVNNV--PVGPKRELESV		VGCNTVDDPLNRS	371	
BCAPN3	DEVPP--	-KGEVKVLRNLPWQGOVENNGSMSDRKWDMSVSDVSKFDKEARLHQ(VQTEDEGFWMSTEDYF1YHFTKLEICNLTDALQSDQKLTQTMVSVN--EGRWVRG	-CSA	444	
BCAPN9	DOVSF--	-RQQLIELIRRNPMQGWEENNGSMSDSSPEWRSVGPABQKRLCITALDGEFRAFKDFKAHFDFKVEICNLTDALQSDQKLTQTMVSVN--QGSWVRG	-STA	364	
BCAPN1	KOVNY--	-RGQVVSLLIRMNRPNGEVENTGAMSDDSSSEWWNDVDPYERDQLRKVMK--DGEFMSFRDFMRFRTRELEICNLTDALQSDQKLTQTMVSVN--EGTRWRG	-STA	381	
BCAPN2	EEVES--	-NGSLOKLIRLRNRPNGEVENTGMRNDNCPSWNTIDPRTKEELTRRME-DGEFMSFSDFSDFLQYSRLEICNLTDALQSDQKLTQTMVSVN--DGNRWRG	-STA	371	
BCAPN8	EEVNF--	-HCRPEKLIRLRNRPNGEVENWSGMSDNAPENYIDPRRKEELDKKAE-DGEFMSFSDFSDFLQYSRLEICNLTDALQSDQKLTQTMVSVN--NGRTRG	-STA	371	
BCAPN10	RELQG--	-QAGQCTILLRQNPWGRRCWQGLMREGGDGSQDAAVASELLSQLQ-EGEFVWEEEELPREFDLETVGYPVTEAHLQSLYTERLICHTRALPGAWVKG--QSA	352		
		Domain II		Domain III	
BCAPN5	CCGCIHKEDTFFQNPQYIYFVKKPED--	-EVLCIQQRPKSTRREKGKGENLAIGFDIYKVE--	E--NRQYRMISL	-QHKAASSTYINSRSVFLRTDQFECRYVIILT	472
BCAPN6	CCGCVNDRFTFLQNPQYIYFVPPEDGH--	-KVMISLQKDLRTYRMRGPDRNYYIIGFELFKV--	H--NRRFRLHBLTQGERAGTSTYDRTVFLSKWLKKGSYVLPVT	472	
BCAPN3	CCCRNPDTWTPWNPQYRKLKLEEDD--DPPDSEV-ICSVLVALQHKNRKRDRKLG-ASLFTIGFAIYEPKEMHGNK-QHLQKDFLFLNASKRSKTYDNKREVSQRFRLPPEYVIVPS	559			
BCAPN9	CCCRNPLDTWTPWNPQYRKLKLEEDD--GQEE--	-CSFLVALMKGQKDRRRLKREG-ANVLTICYAYECP--DXD-EXKNTFLFTHASRASRKFVIRLREVSDFRLPPEYVIVPS	471		
BCAPN1	CCCRNYPATFWNPQYRKLKLEEDD--DPPDSEV-GCSFLVALMKGQKDRRRLKREG-RDMEHTGFAYVEVLPQAVHLKDRFELFLAARSARSOEFLINREVSTFRFLPPEYVIVPS	499			
BCAPN2	CCCRNYPATFWNPQYRKLKLEEDD--DEEDGES-GCTFLVALMKGQKDRRRLKREG-RDMEHTGFAYVEVLPQAVHLKDRFELFLAARSARSOEFLINREVSTFRFLPPEYVIVPS	487			
BCAPN8	CCCRNYPATFWNPQYRKLKLEEDD--DEEDGES-GCTFLVALMKGQKDRRRLKREG-RDMEHTGFAYVEVLPQAVHLKDRFELFLAARSARSOEFLINREVSTFRFLPPEYVIVPS	487			
BCAPN10	CCCRNNS-SGFPSPNPKFLRVSEPVYIYLQSRSLHAADWGRARALVGDSHTSWSPASIPGKHYQAVGLHMKVKEKRRVNLPRVLSPMPPVAGTACHAYDREVHLRCELSPGYYLAVPS	471			
		Domain III		Domain IV or T	
BCAPN5	TTEPCHGTGEPLLRTVDPVSNCRELRLDEPHK--		C-	-MSSLOGYPQLVTVQHVGLAGLK--	530
BCAPN6	MFOHGRTEFLRFLFSEAPVQVLRELTQDMKMS		C-	-MHLARYDQVTVQTVTVAEGLKXY--	532
BCAPN3	TTTEPHQEGEFLRFLFSEKRNALSEEVENTISVDPVKEKKKTFLIIFVSDRANSNEELGVDQESEDGEGKTSQDKQSQPQPGSSDQESEQQAFRFLFKQIAGDOMECADELKVLAT		E-P	-PKPTP--PQDETEEDRFLRFLFVQGVEDKETAEELLEYVINA	673
BCAPN9	TTTEPHQEADFLCRLIFSEKRAITRDHGNDVDDLP--		E-P	-PKPTP--PQDETEEDRFLRFLFVQGVEDKETAEELLEYVINA	548
BCAPN1	TTTEPKEGDFVLRFLFSEKSAZGTVELDQOQANLP--			-DEQVFLSEEEIDENFKFLFRQLAGEDKELISVKELTILRN	572
BCAPN2	TTTEPKDQGDFCIRWFSEKKAQYQADDETEANL--			-EEFDVLSSEEEIDENFKFLFRQLAGEDKELISVKELTILRN	559
BCAPN8	TTTEPKDQGDFCIRWFSEKKAQYQADDETEANL--			-EPHPDMDDEEDEHVRSEFLFEEFVGDRDSEISIANQLKRVLINE	562
BCAPN10	TELKDAPGEFLRFLFSTGRVSLSAIRARAVRNTT		G-	-AALPAGENCTVQLRGSRWVRCQTAGGSRFNASYPTN	341
		Domain IV		Domain V	
BCAPN5	--SPTGANSVIIKCEGDKVRSVAVQKG--	-TSTPEYINVKGICPYRKKLQSOPITVQVWHR--		-VLFDEFILQGVHLKADPDONLQALIHLRDRNSRQ	618
BCAPN6	--ANETVNPYLIKQCKREKQVPSVQK--	-TVHAFSTDQALFQYRRTIDTIPIIIQVWNSR--		-KFCQFLQVLTDAQPSDCRDLKSLYLRKKGPT	620
BCAPN3	VVNNKHDLLRTKHTGFTLSECRSKLIALHDTDGSGKLALQEFHHLWKKTAKAQLKFLKTYDTCGCTINSTYHNAVNADGFLANNQLYDIXTHYADKRRHIDFDSFICCPVRLCEMFRHAF	799			
BCAPN9	VLQKKEDLIFKFLSLISCKNIIISLMDTSGNKLEFDEFKVFWDKLLQMINLFLRDAOKSGTMSTYELTALKAAGFQLSHLLQQLVYADEEQLQDFDIFNCFVCLVRLLETMRFFKTL	692			
BCAPN1	IZSKHHDLLRTKHTGFTLSECRSKLIALHDTDGSGKLALQEFHHLWKKTAKAQLKFLKTYDTCGCTINSTYHNAVNADGFLANNQLYDIXTHYADKRRHIDFDSFICCPVRLCEMFRHAF	679			
BCAPN8	VLAQKTDKISQDGSIETCKVMDLSDGSDGKGLKAEFYIILATKQIYQKLYREIDVDRGCTGYNSTEMRKAEEAGFQHPCQLHQHVIVARFADDQILIDFDFNFRVCLVRLLETFLKIFKQL	682			
BCAPN10	--PCPFPSVPEGPGPRCVRITLQHCRPSD--	-TEPHPIGFLIPQVPEGGRSQQDAPPFLQEPILLSCPVPHRYAQEVSRUCLLPAQYTVKVVPSYLEDECAFVTLATRDRPSIHSQ	654		
BCAPN5	PSNLPGTVVHILSSTSILMAV- 639				
BCAPN6	AKVKQGRHSFVKVSISSDOLTEL- 641				
BCAPN3	DDGDGQIICKLNVLWLOLTHMYA 821				
BCAPN9	STKQKPEIHLNINEFIHLTMNI 690				
BCAPN1	DTDLDGCVVTEFLDFKWLQLTMAPA 714				
BCAPN2	DEPENTGTYEFLDLISLWCSFVSL 700				
BCAPN8	DDKQNGTIVLQVNLNANLCCVLU 703				
BCAPN10	EMLQOFLQEVSVMAVMRT 672				

FIG. 5

PERCENT IDENTITY

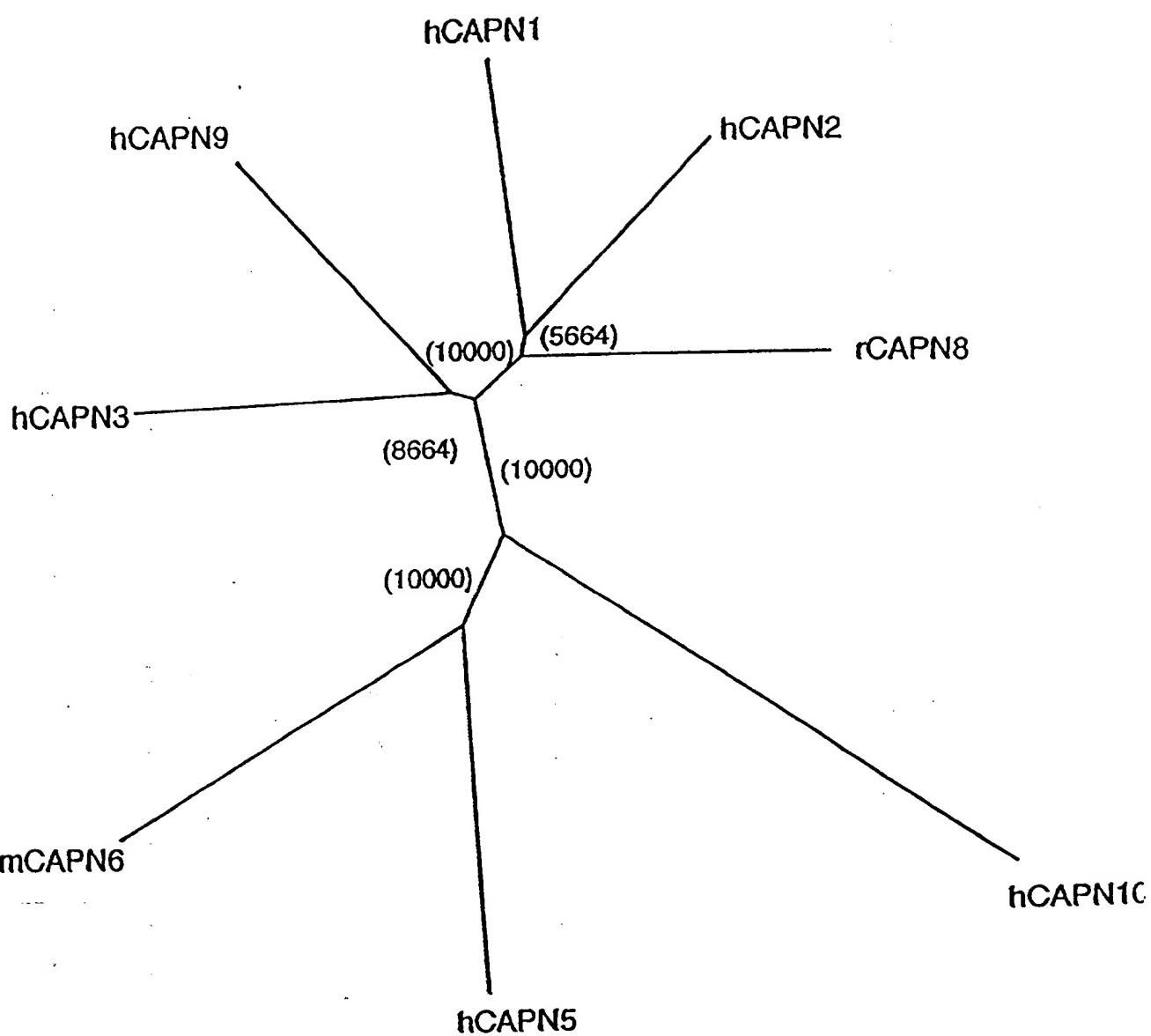


FIG. 6

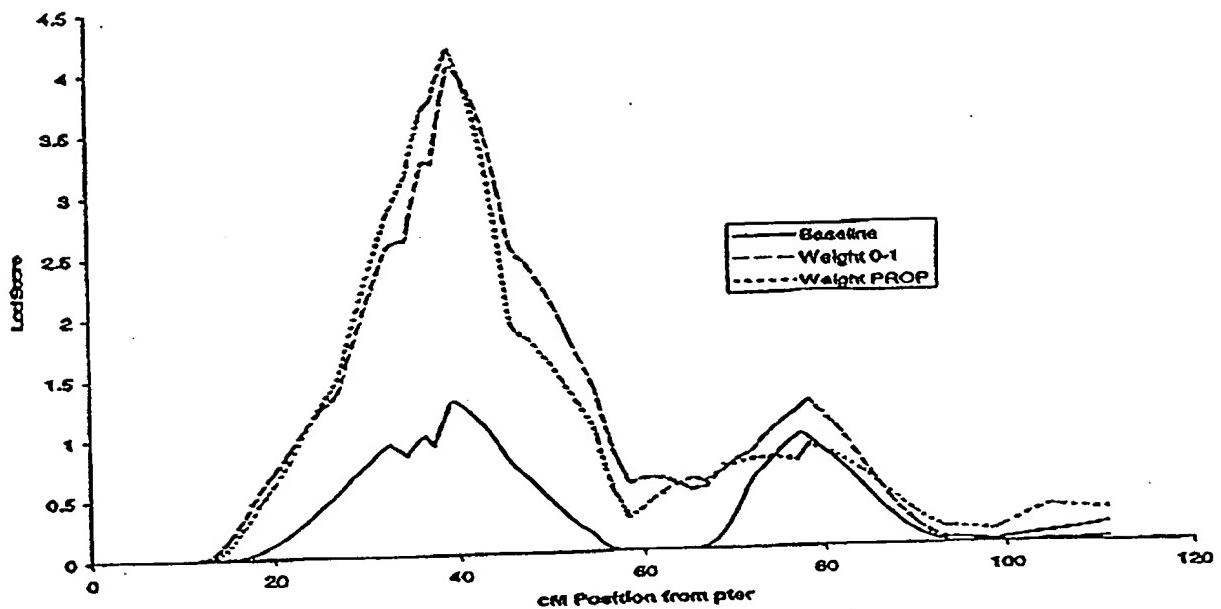


FIG. 7A

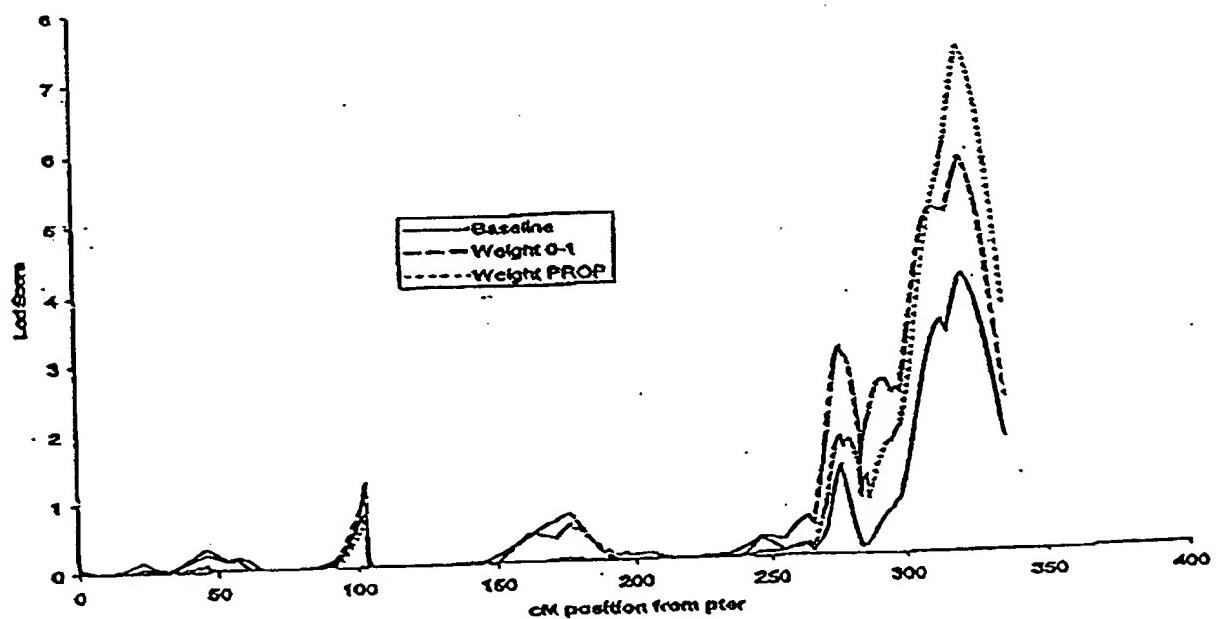


FIG. 7B

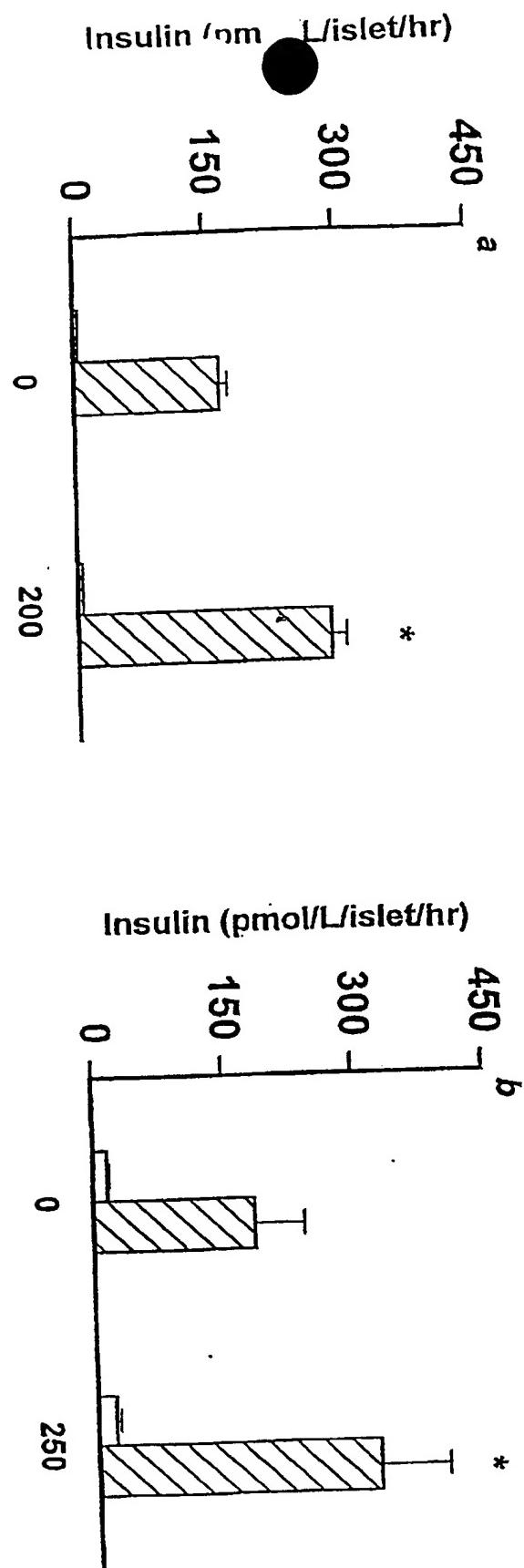
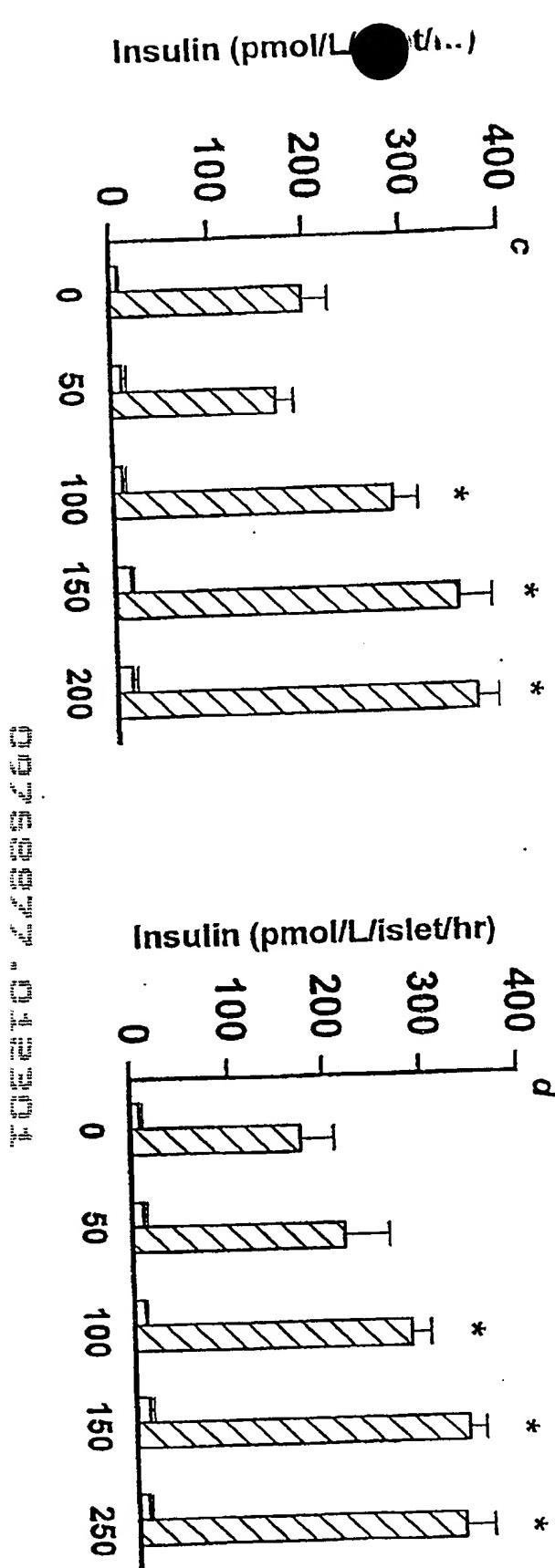


FIG. 8

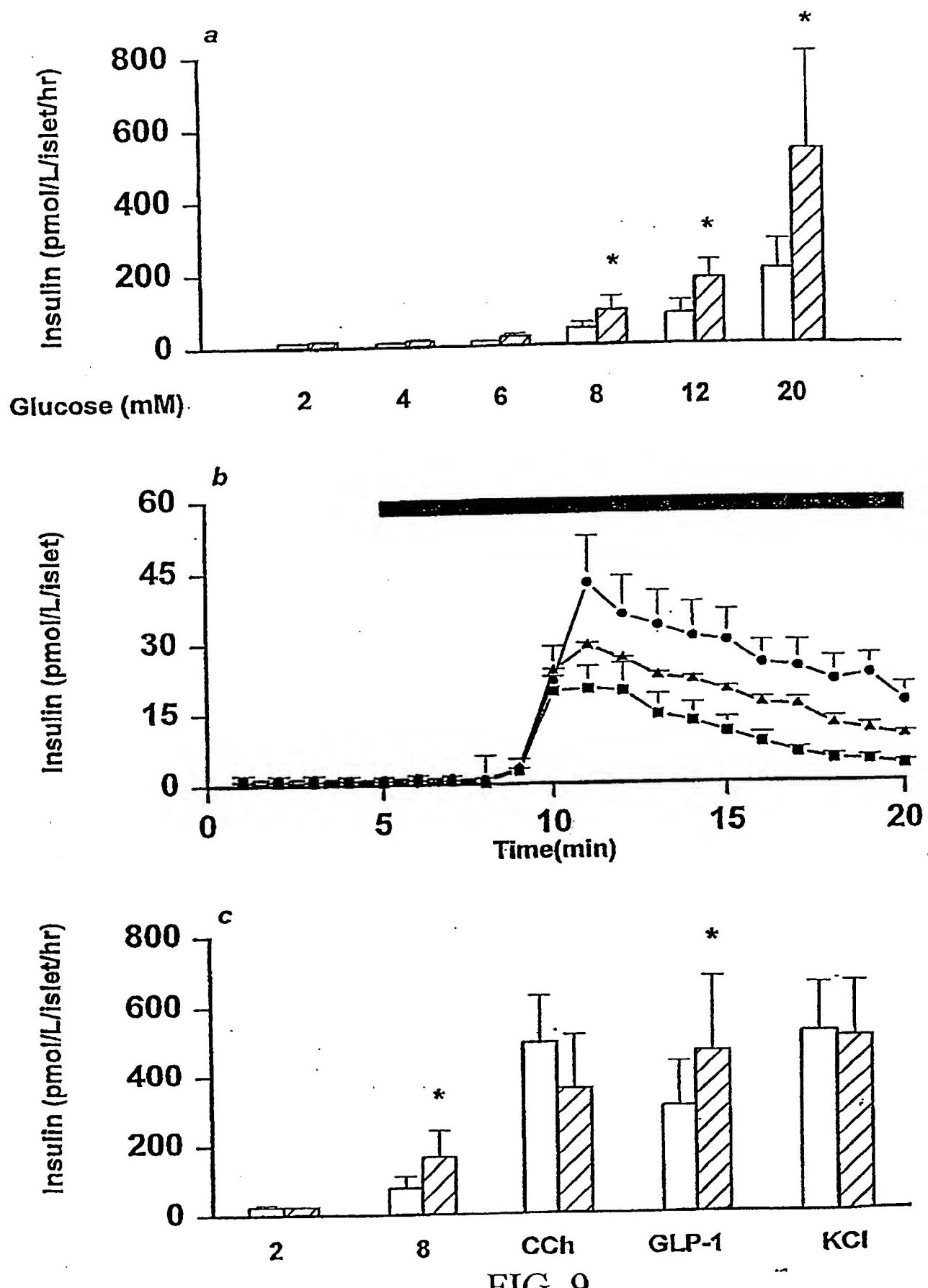
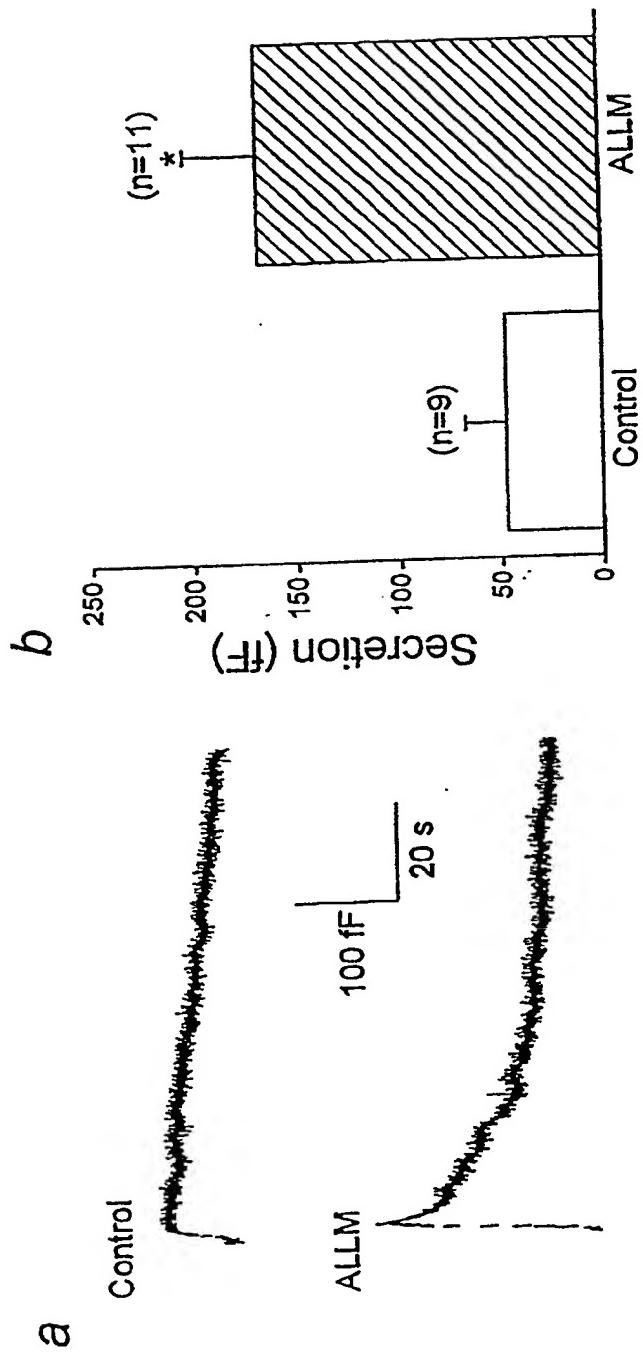


FIG. 9

FIG. 10



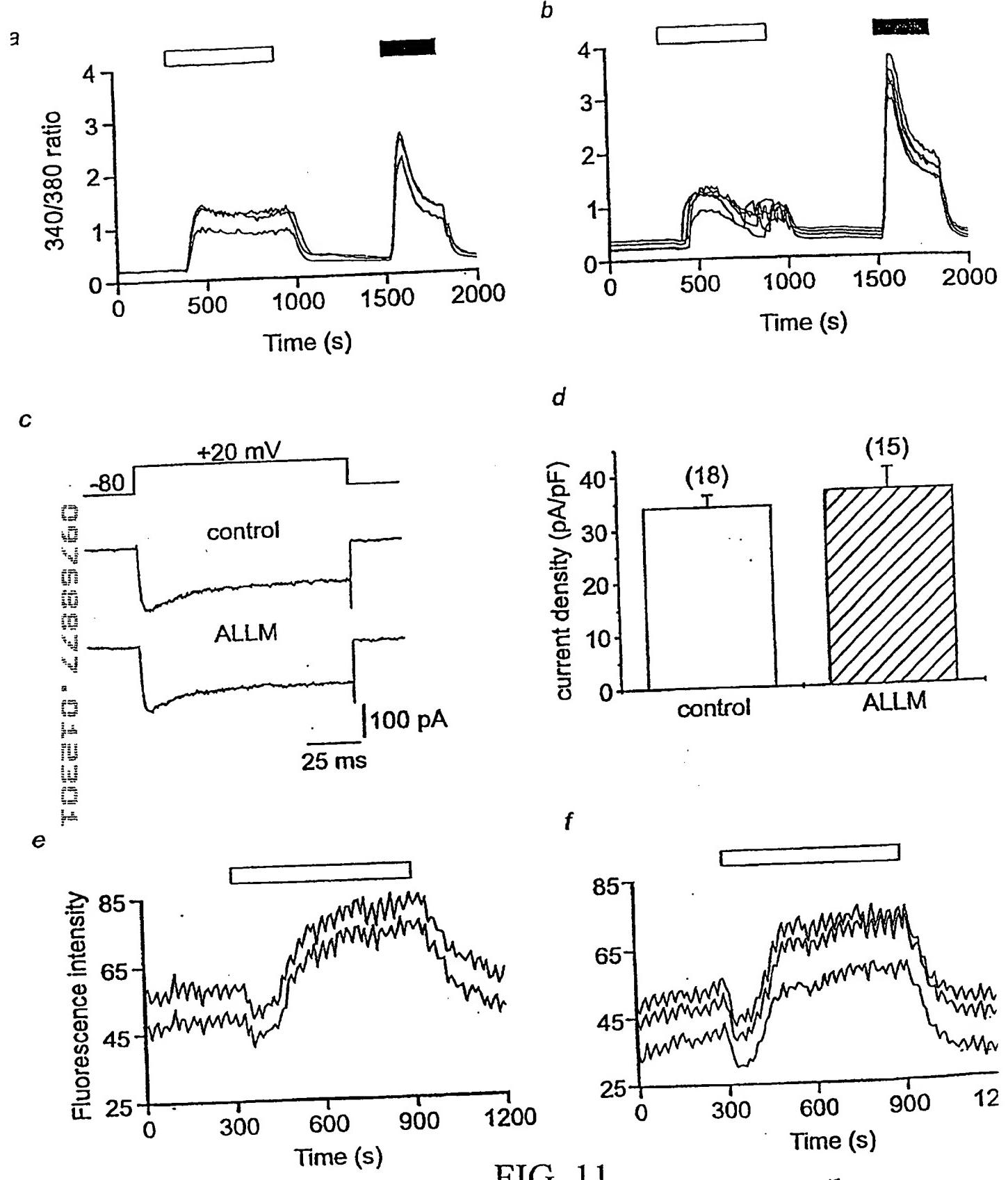


FIG. 11

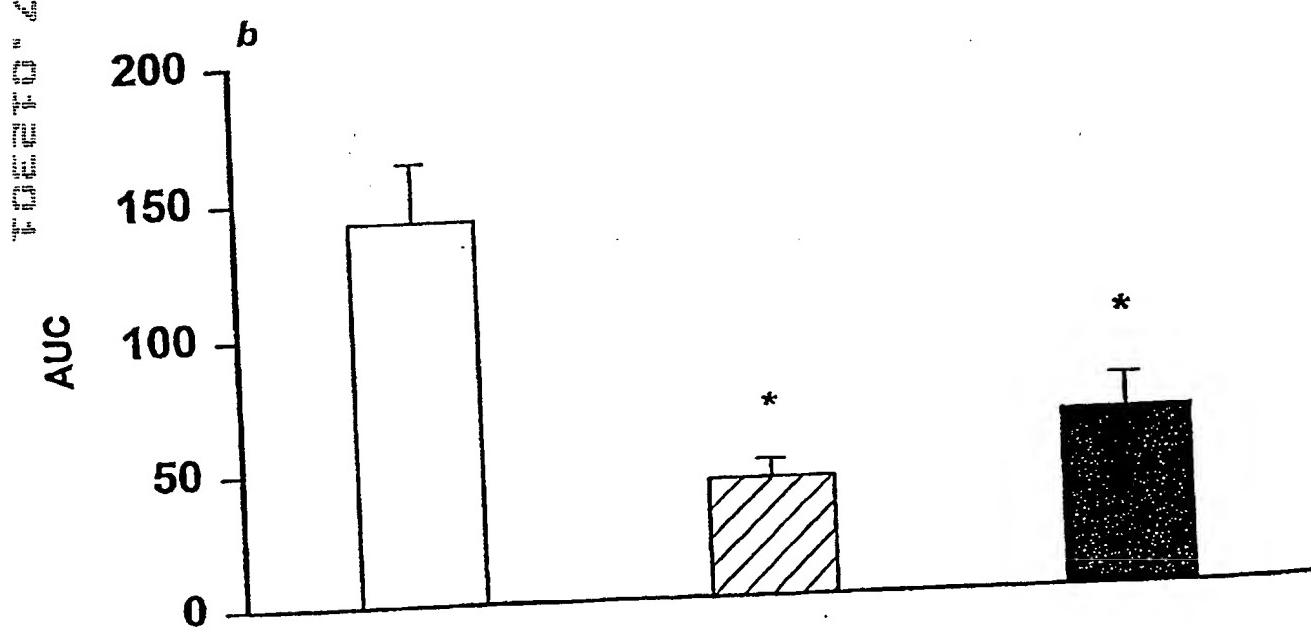
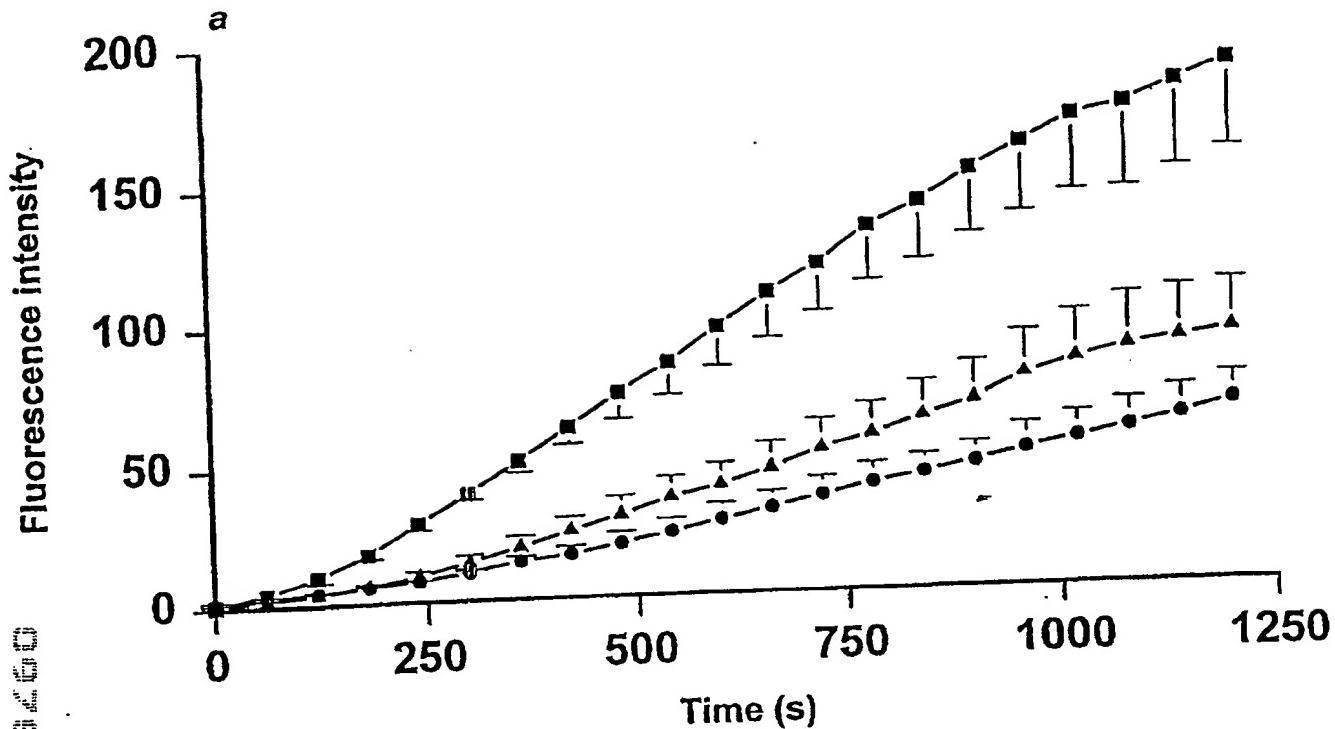


FIG. 12

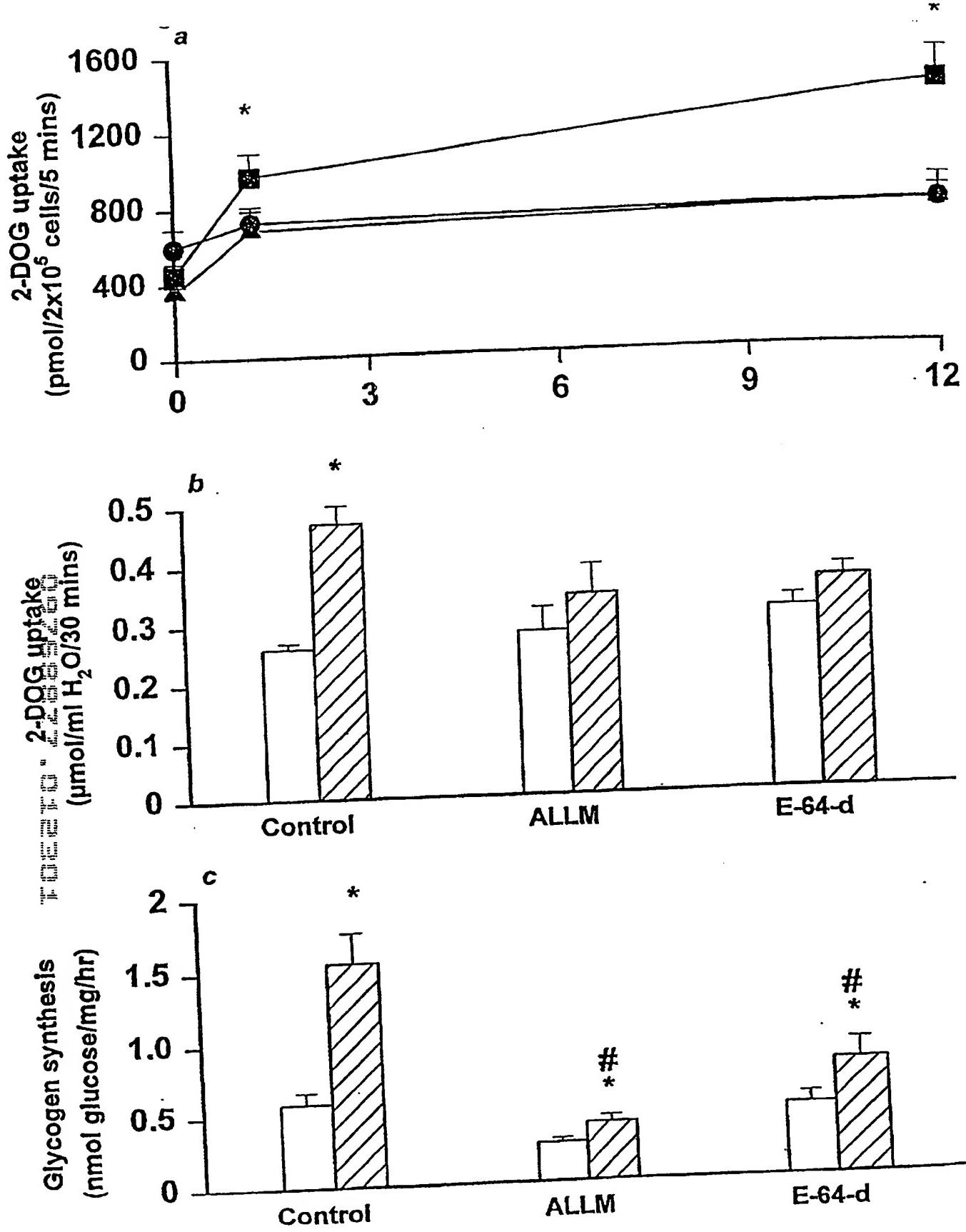


FIG. 13

Fig14. Effect of 48 hours exposure of islets to calpain inhibitors on insulin secretion

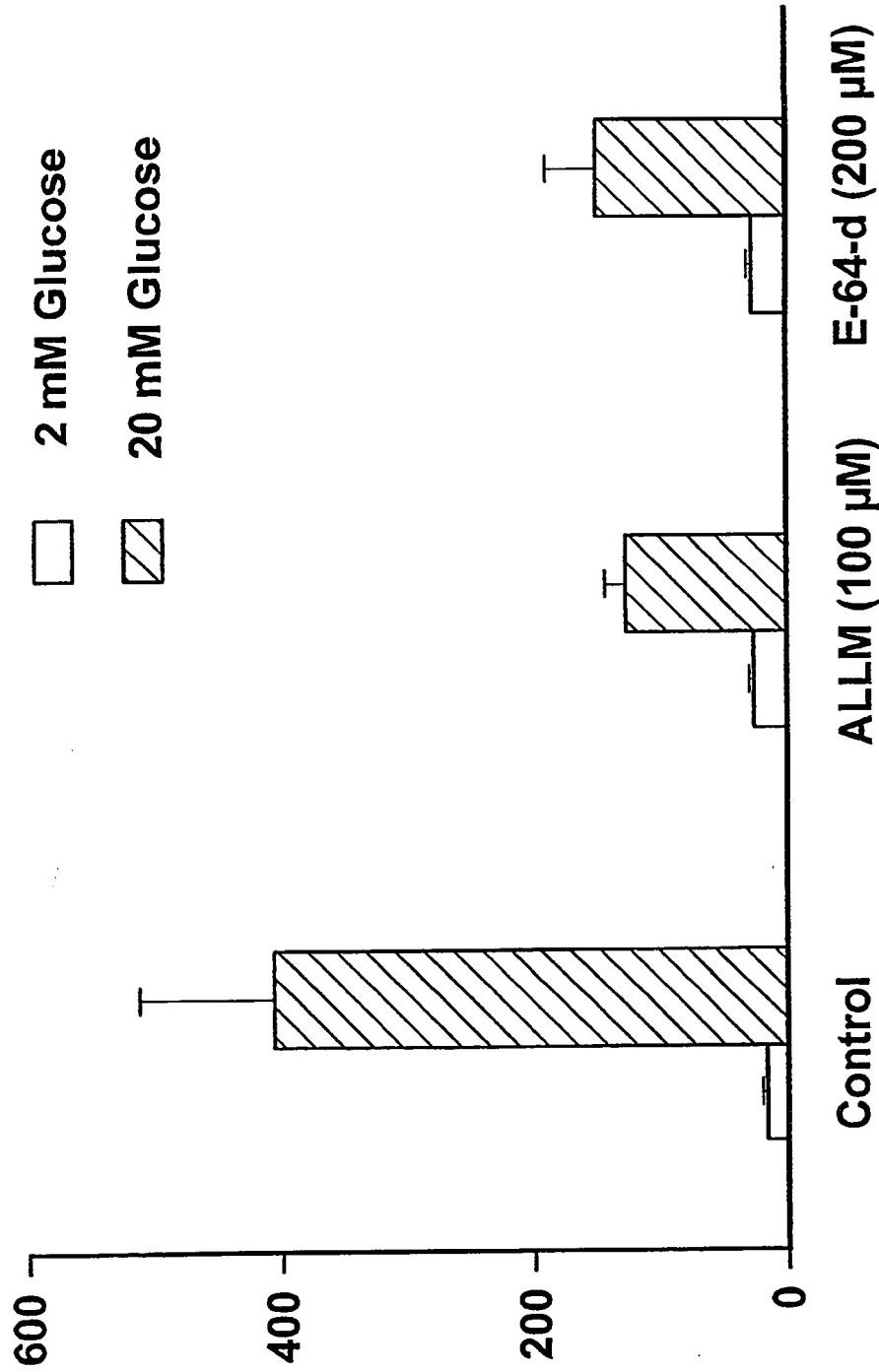


Fig 15 Insulin content in 48 hour cultured islets (n=4)

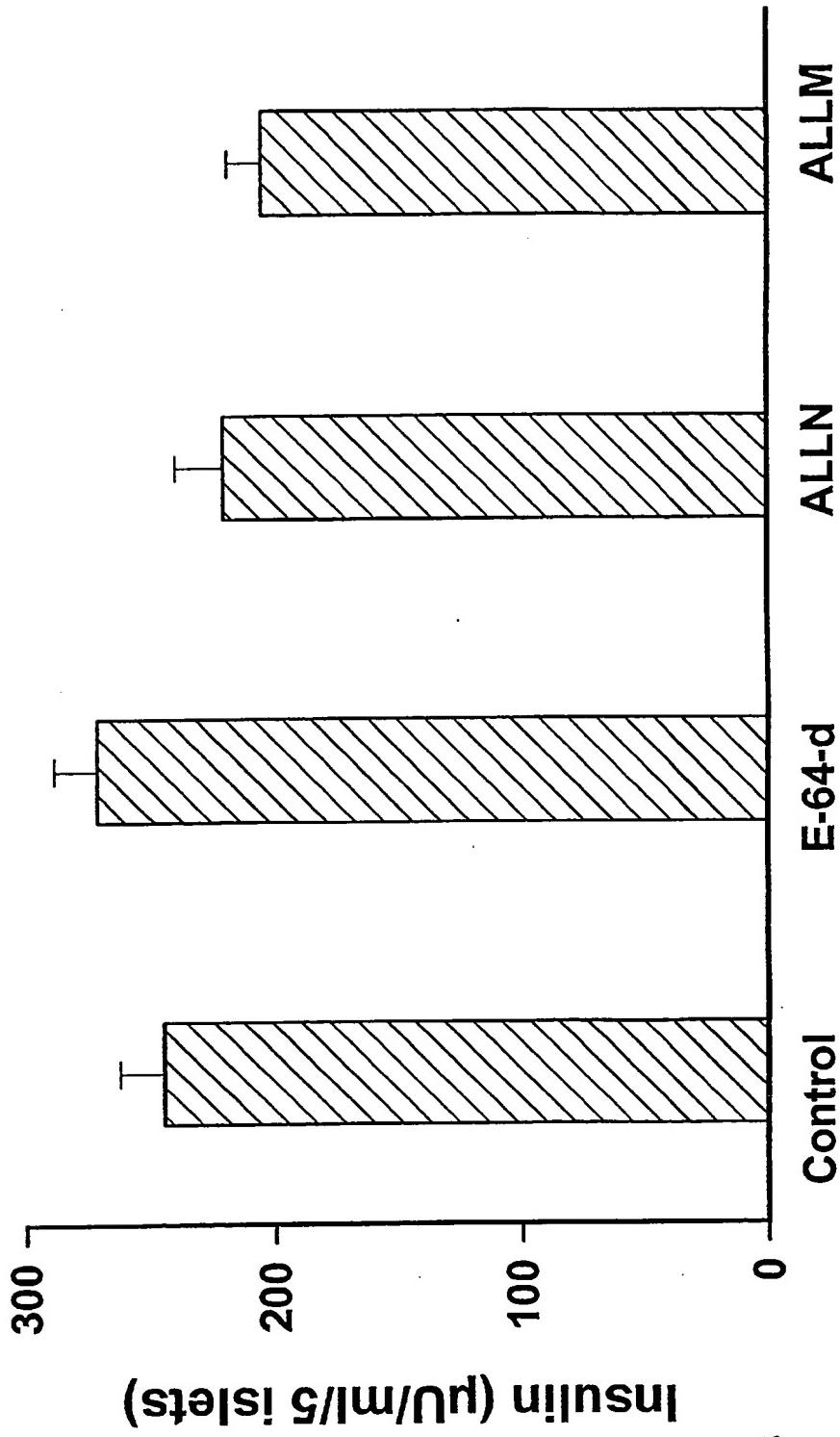


Fig16. ALLM dose response in 48 hour treated islets

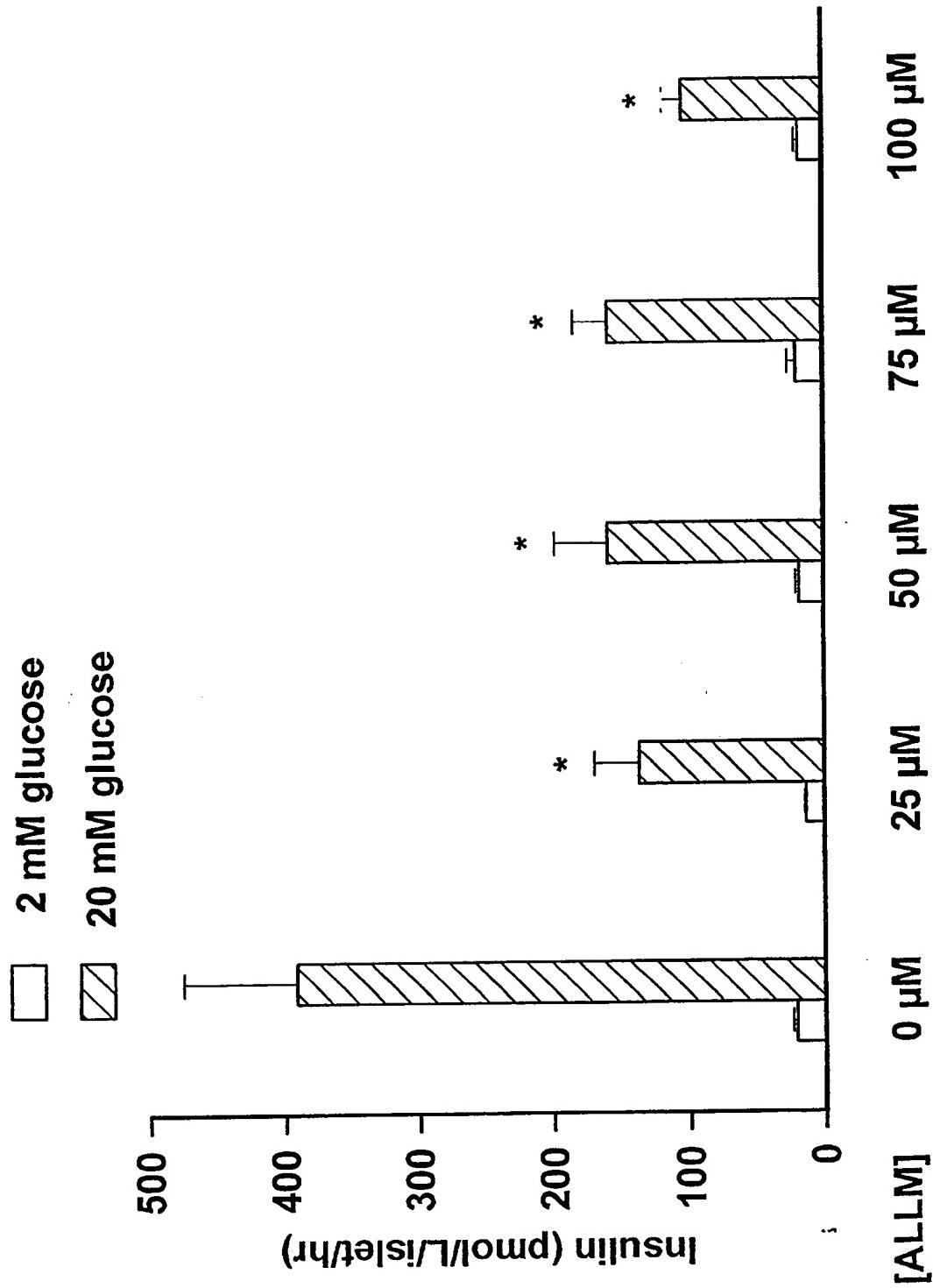
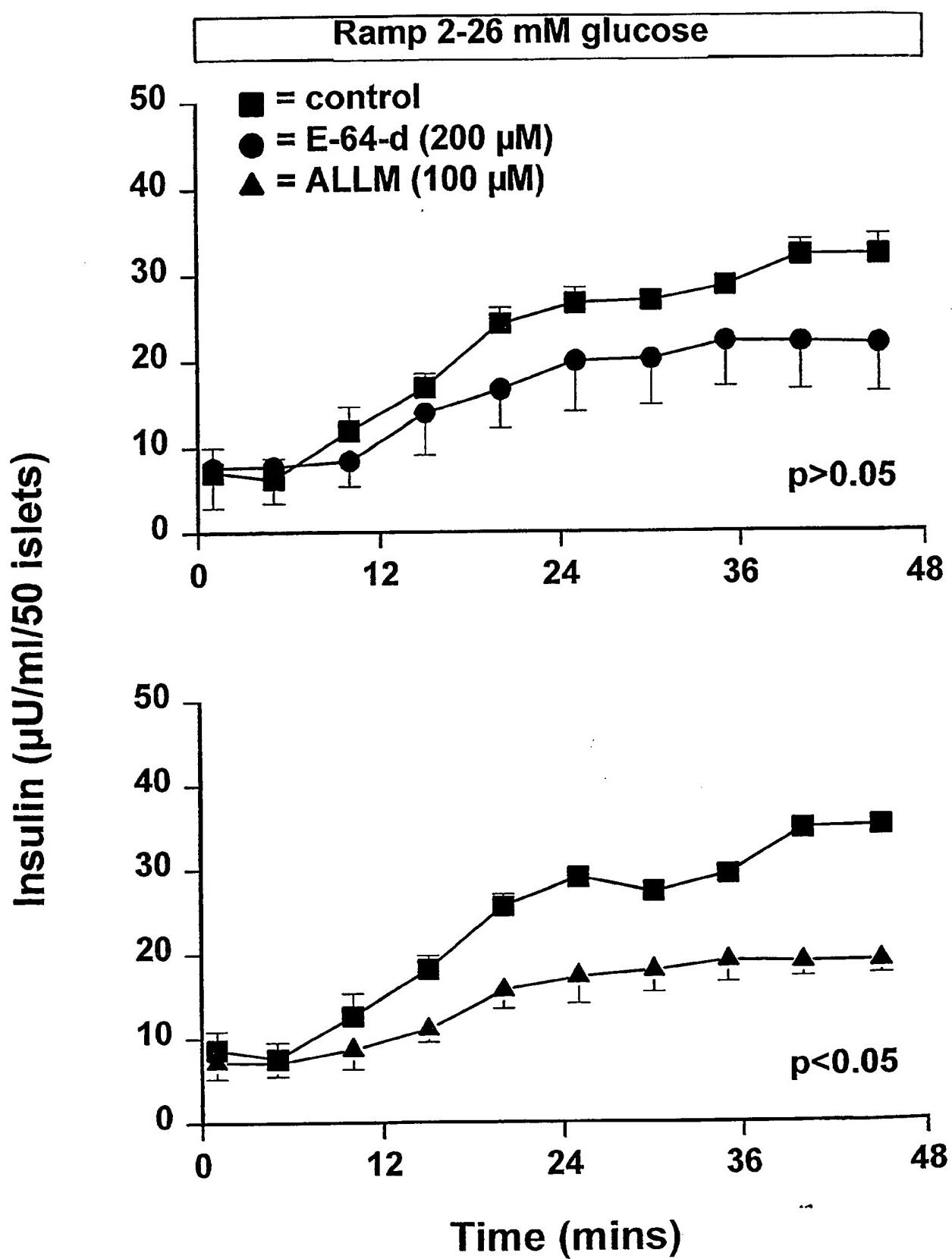


Fig17 Perifusion of 48 hour cultured islets (n=4)



Insulin secretion in ALLM or E64-d treated mouse islets: Reversal study

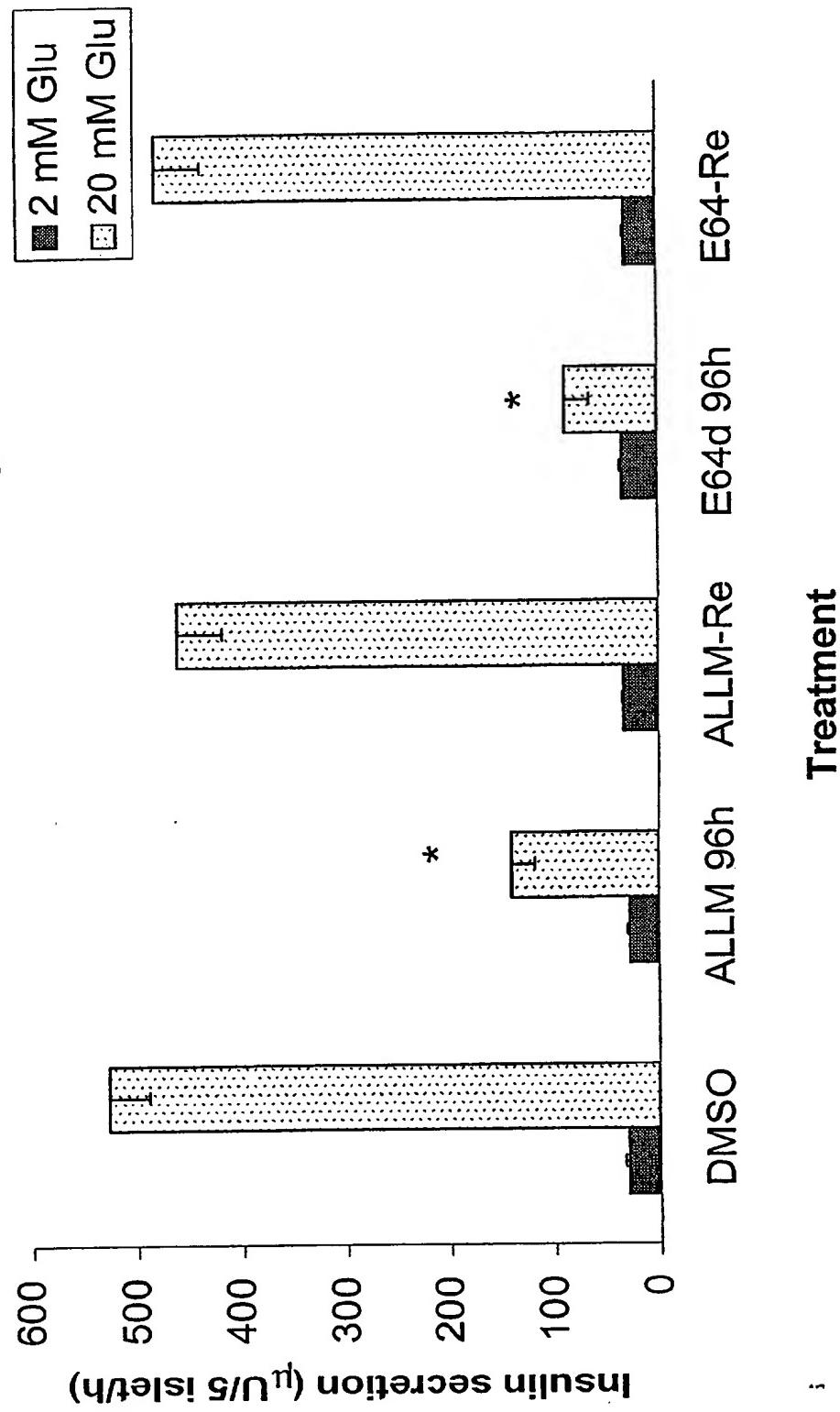
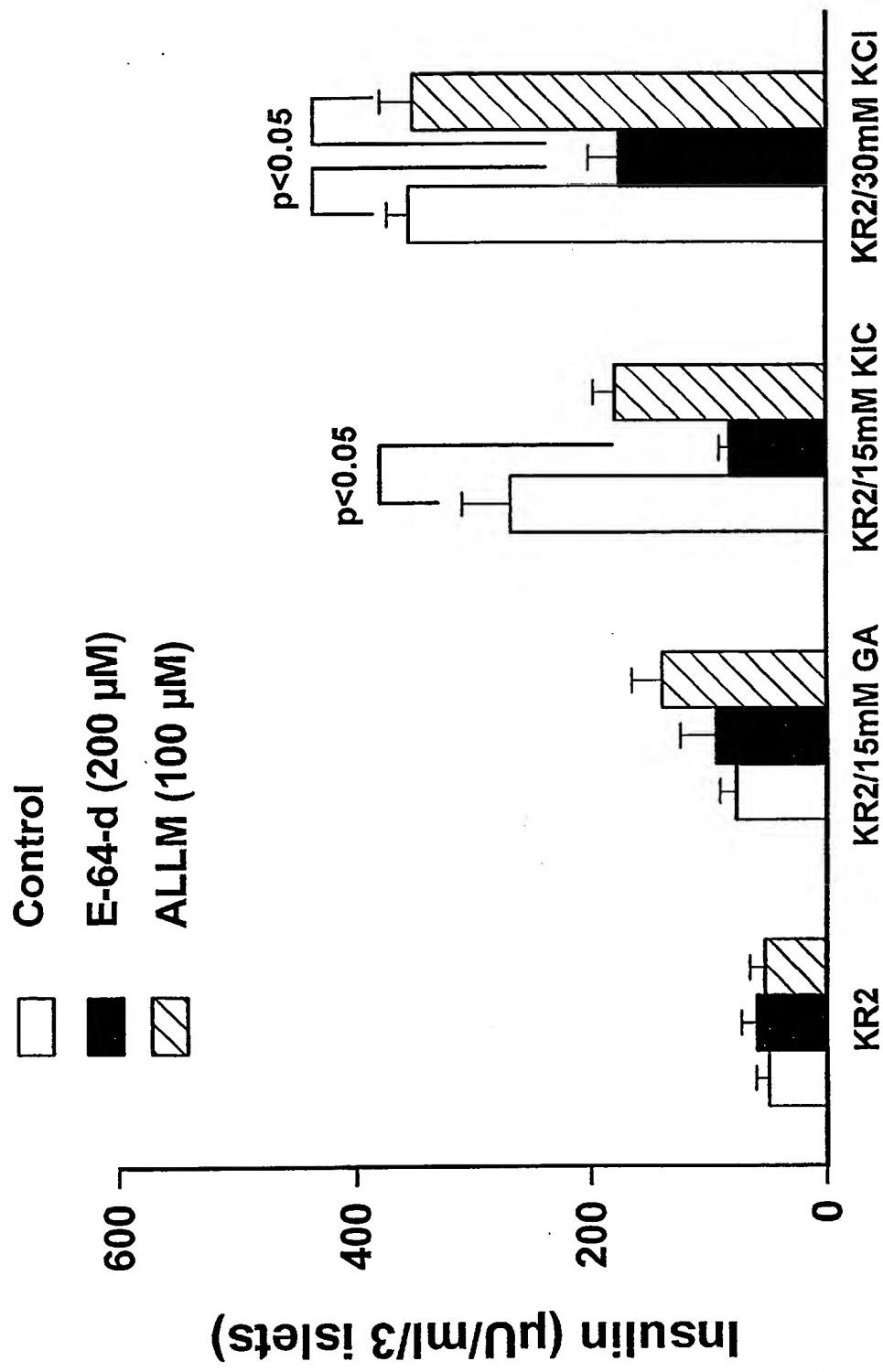


Fig. 18

Fig 19. Insulin secretion by islets following exposure to calpain inhibitors for 48 hrs

(n=6)



Mastoparan-induced insulin secretion:
Effects of calpain inhibitor

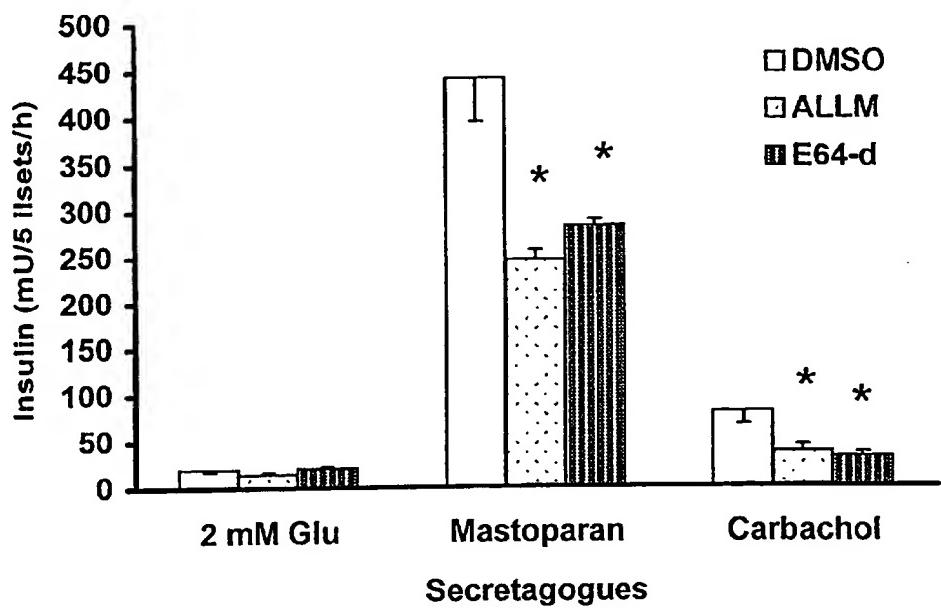
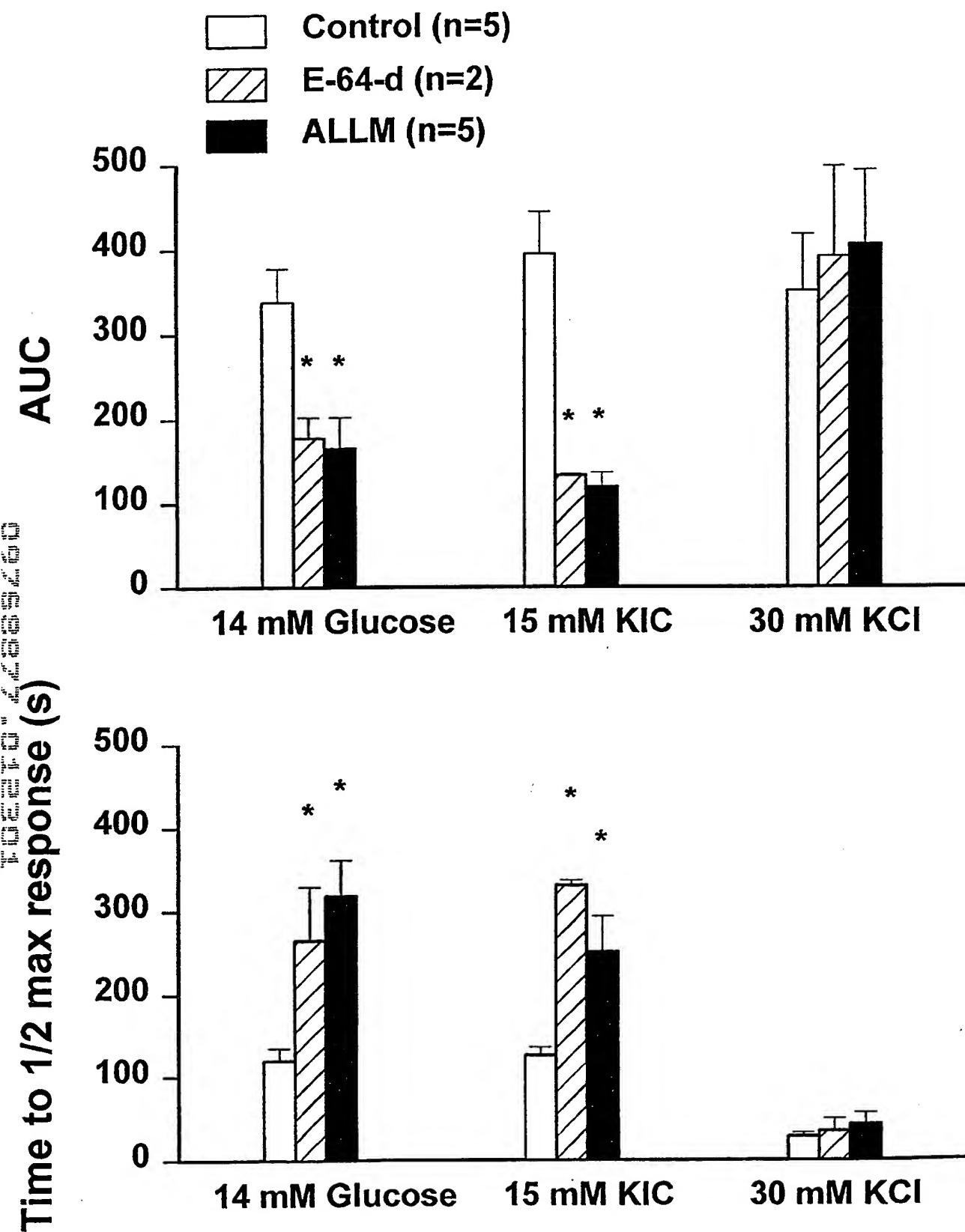


FIG. 2D

Fig 21. $[Ca^{2+}]_i$ responses to glucose, KIC and KCl



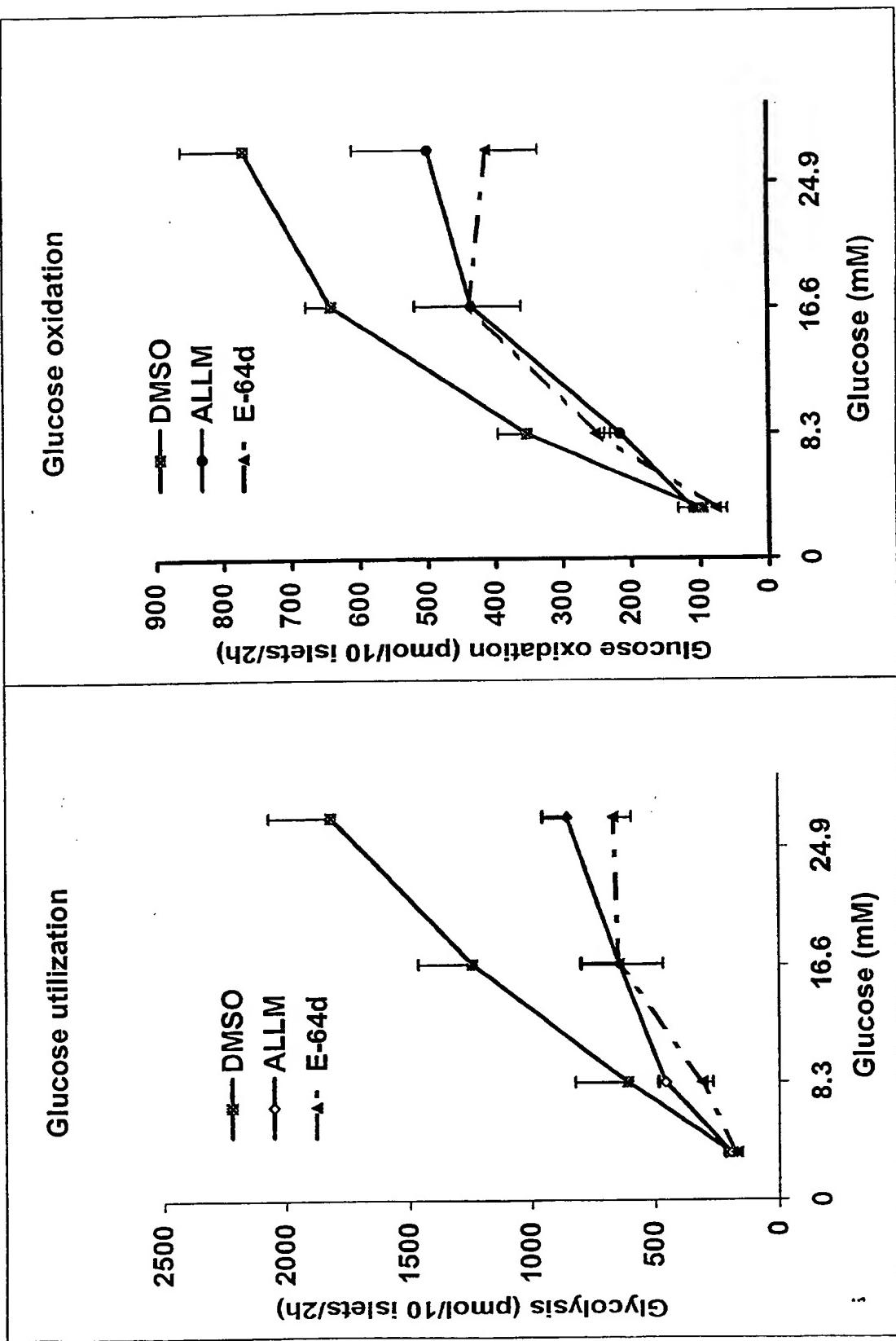
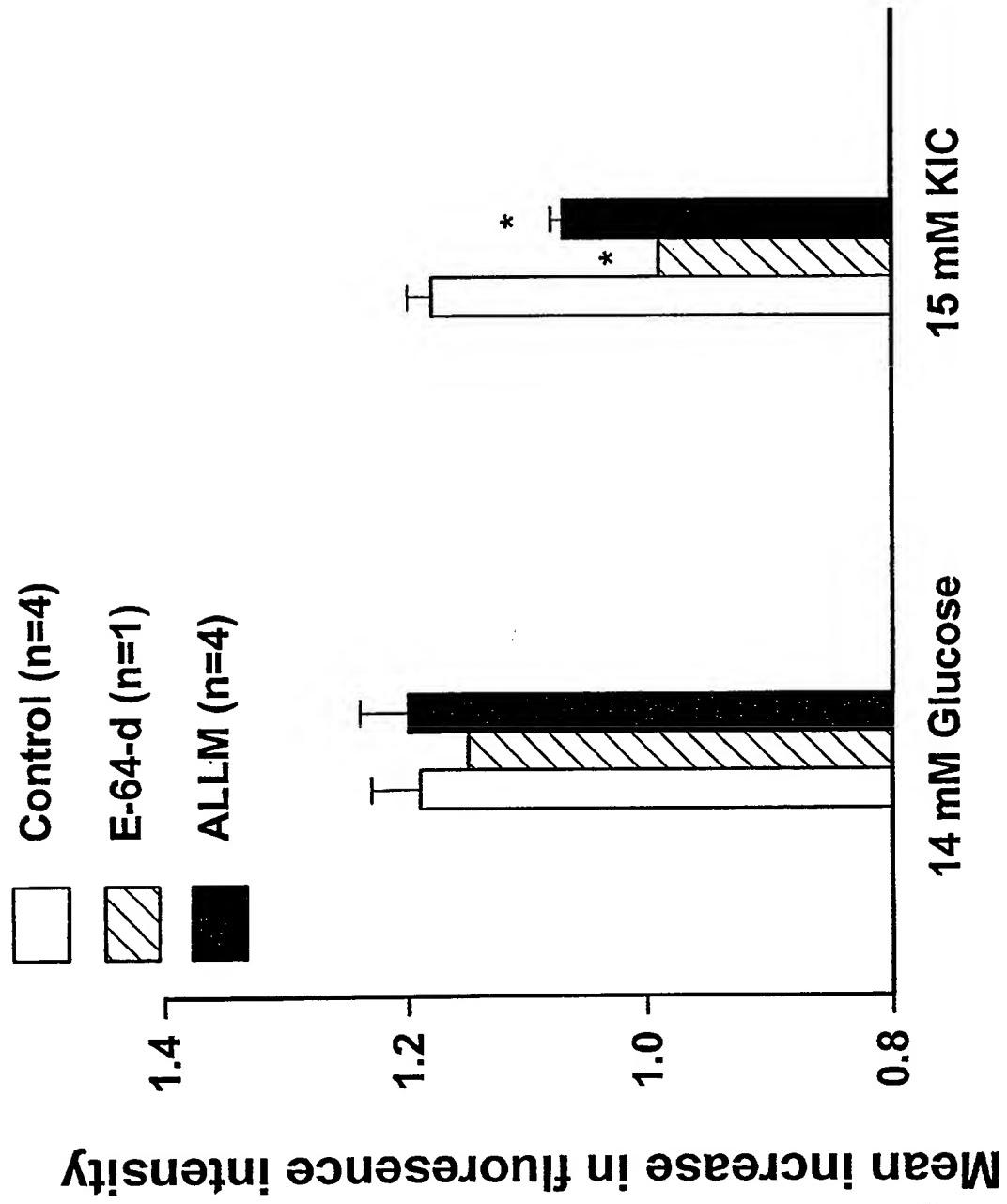


Fig.22

Fig 23. NAD(P)H responses to glucose and KIC



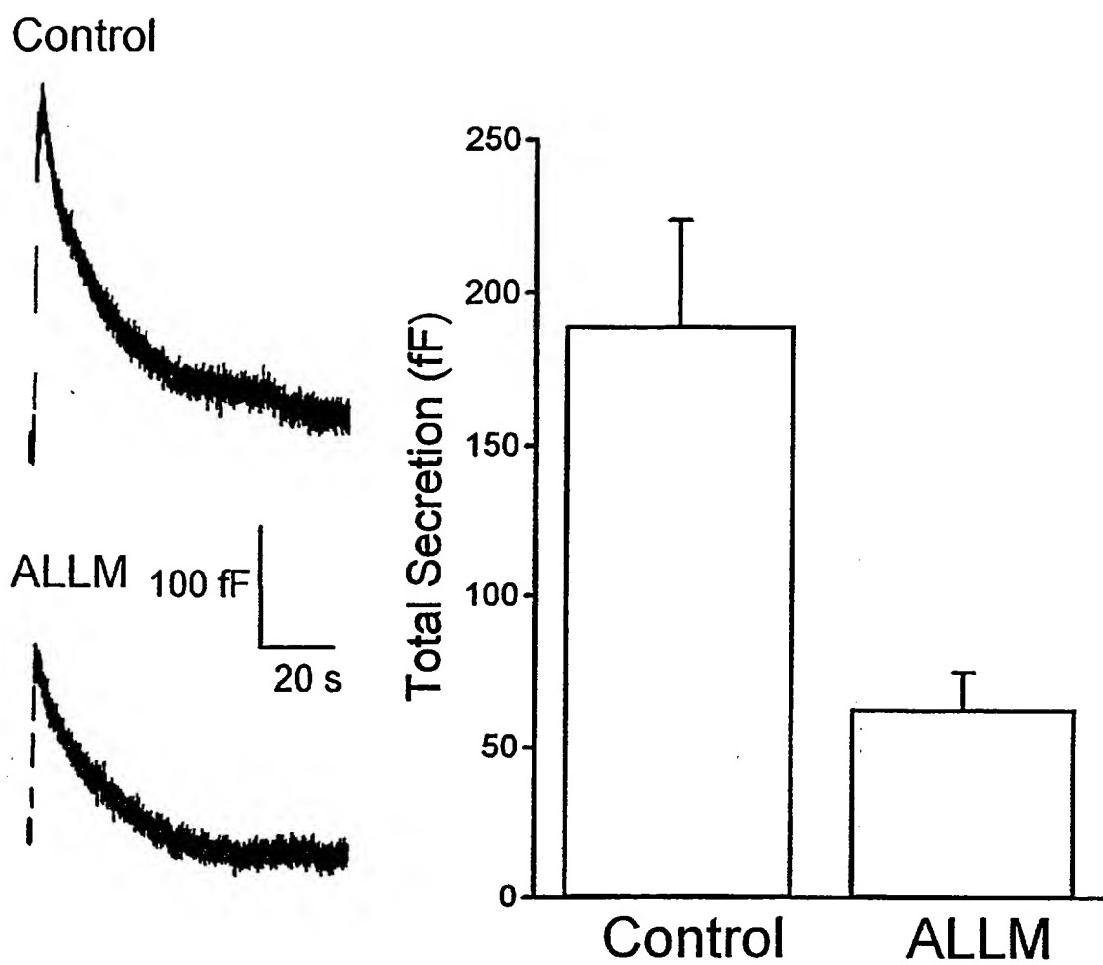
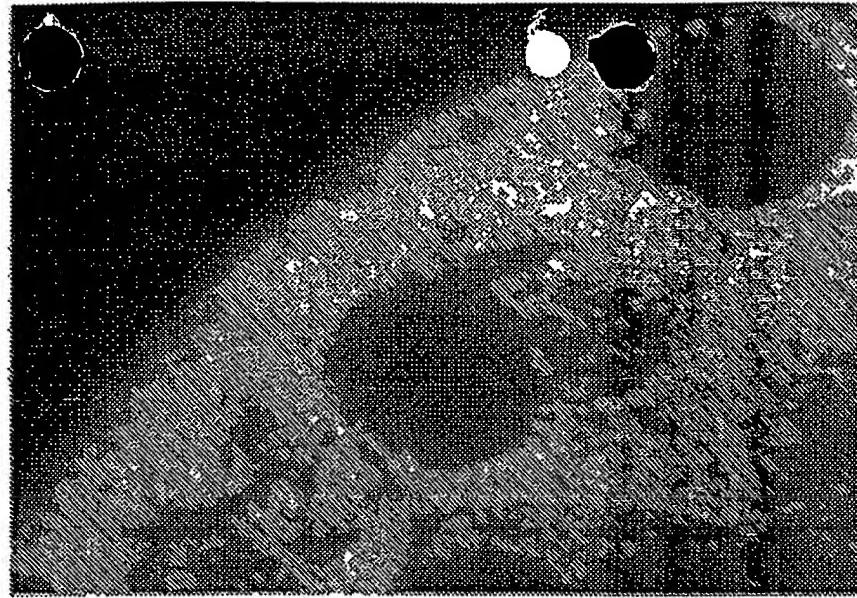


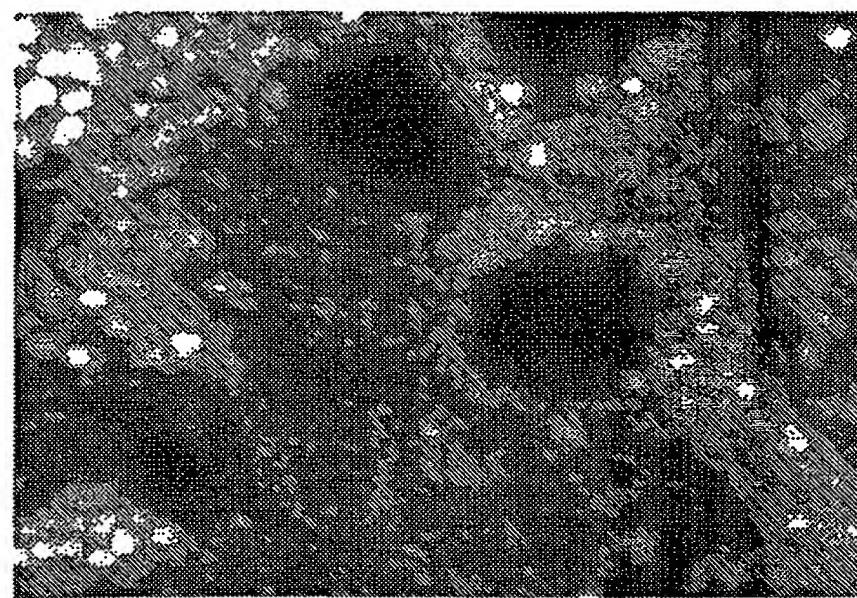
Fig. 24. Measurement of membrane capacitance in isolated β -cells

2020-07-01 22:29:26.0

DMSO



E64d



ALLM



FIG.25

Calpain activity after 48 treatment with ALLM or E-64d

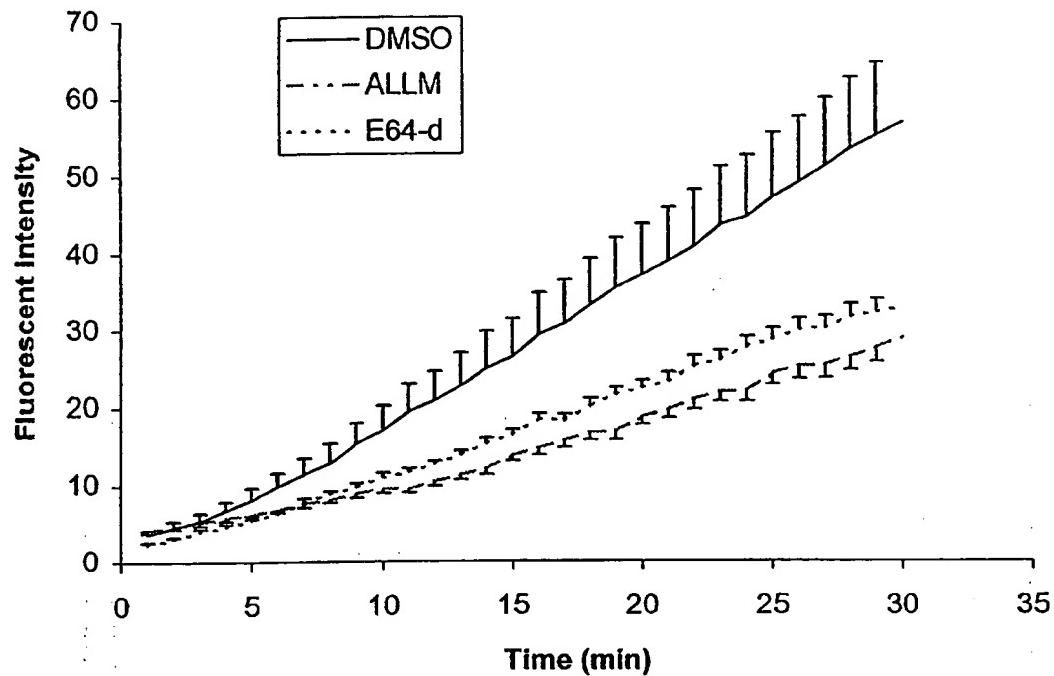


FIG. 26